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Complex rearrangements involving der(8)t(8;20) and der(14)t(8;14)t(11;14), *CCND1*, and duplication of *IgH* constant region in acute plasmablastic leukemia

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Abstract We report on a rapidly fatal case of acute plasmablastic leukemia in a 72-year-old male from The Ukraine, who was 70 km away from Chernobyl at the time of the atomic accident in 1986. Spectral karyotyping and fluorescence in situ hybridization (FISH) studies of a bone marrow sample obtained at diagnosis revealed a hypodiploid karyotype with 45 chromosomes and two novel complex rearrangements, der(8)t(8;20)(p11.2;p?12) and der(14)t(8;14)(p?;p11.2)t(11;14)(q13;q32), with juxtaposition of CH (constant region of *IgH*) sequences to the oncogene *CCND1* (translocated to 14q32). FISH analysis demonstrated that the CH on the der(14) was duplicated. © 2006 Elsevier Inc. All rights reserved.

1. Introduction

Primary (de novo) plasma cell leukemia (PCL) is a rare hematologic disorder that accounts for less than 5% of plasma cell malignancies. Kyle et al. [1] proposed the following criteria for the diagnosis of PCL: greater than 2×10^9/L circulating plasma cells with a plasmacytosis of more than 20% of peripheral white blood cells. Compared to multiple myeloma (MM), PCL displays a more immature phenotype and an array of adverse prognostic indicators, including higher prevalence of extramedullary disease, anemia, thrombocytopenia, hypercalcemia, and renal failure, as well as elevated ß$_2$-microglobulin serum levels, increased lactic dehydrogenase, higher proliferative capacity, a lower overall response to therapy, and a shorter overall survival (3–8 months) [2–4].

The plasma cell is the end product of B-cell differentiation and produces monoclonal immunoglobulin heavy and light chains. The primary chromosomal translocation in plasma cell dyscrasias, involving the immunoglobulin heavy chain (*IgH*) at 14q32, results in deregulated expression of an oncogene [5]. While PCL and MM display similar chromosomal abnormalities, the incidence of such abnormalities is higher in PCL, although the applications of interphase fluorescence in situ hybridization (FISH) and spectral kayrotyping (SKY) have disclosed certain chromosomal abnormalities, including cryptic translocations, in almost all patients with plasma cell neoplasms [6,7]. PCL is also cytogenetically more complex than MM and is more likely to display a hypodiploid pattern and deletions of chromosomes 13 and 17 [3,8,9].

Here, we report on a case of rapidly fatal, acute primary PCL presenting with chromosomal hypodiploidy and complex rearrangements involving chromosomal breakpoints at 8p11.2, 11q13, 14q32, 20p12, and the genes *CCND1* (cyclin D1) and *IgH*, as revealed by SKY and FISH analysis.

2. Case report

The patient, a 72-year-old former steelworker from Kyiv, Ukraine was admitted to the emergency room on August 10, 2003, with acute urinary retention due to prostatic hypertrophy and mild renal insufficiency. Routine complete blood count (CBC) revealed a markedly elevated white blood cell (WBC) count of 50.0×10^9/L, hemoglobin (Hb) of 79 g/L, and a platelet count of 139.0×10^9/L. Differential WBC count showed 41% blasts, prompting a transfer to the Institute of Hematology and Transfusion

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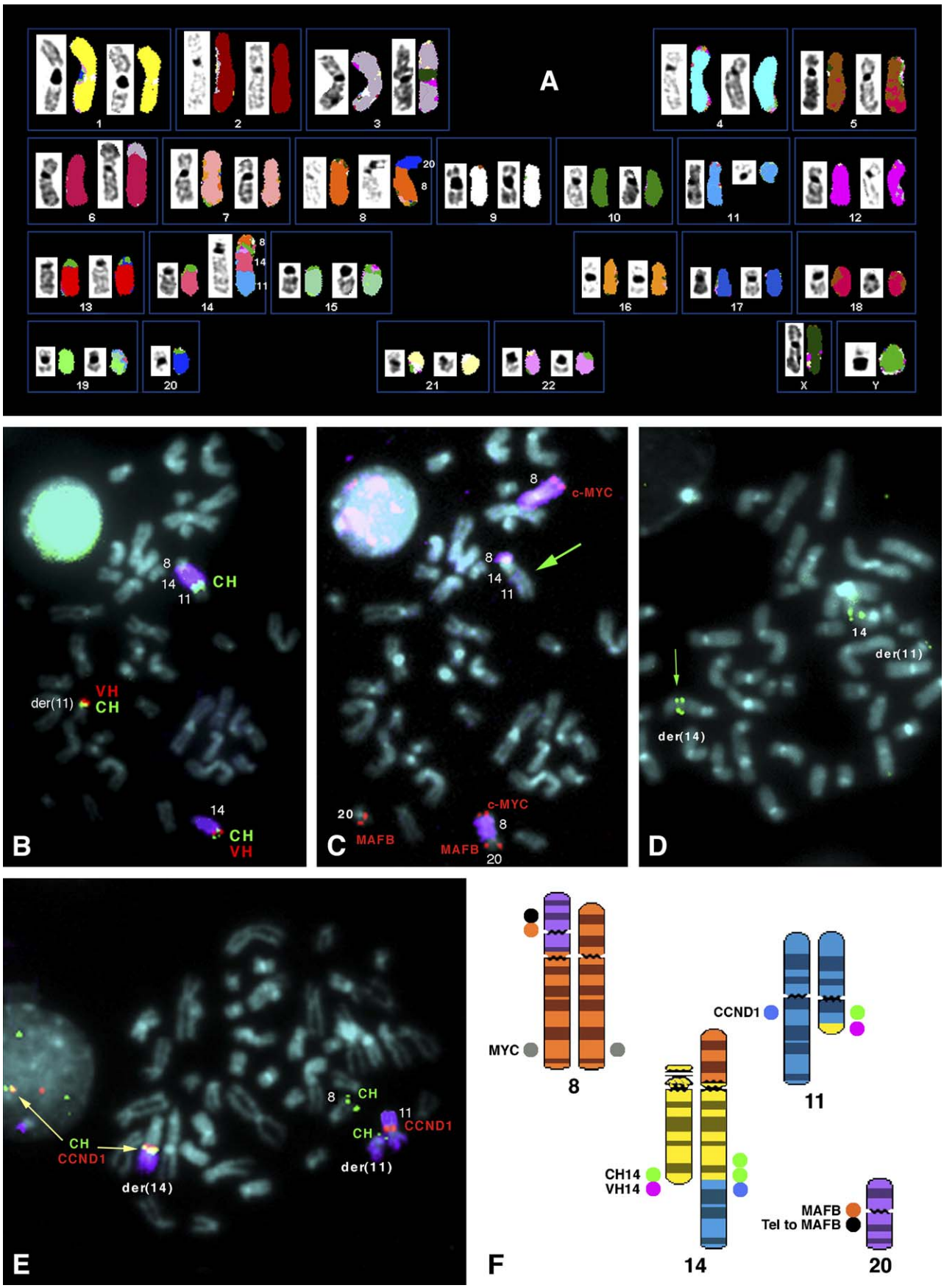
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Medicine for further evaluation. Past medical history was notable for some exposure to radiation in the past: the patient had spent the first 6 days after the Chornobyl atomic accident working outside in his garden, which was approximately 70 km from Chornobyl.

Upon admission to the Institute of Hematology and Transfusion Medicine on August 15, 2003, the patient complained of marked, generalized weakness. Physical examination revealed an elderly man in no acute distress, with a urethral catheter in place. Liver, spleen, and lymph nodes were normal in size, and there were no cutaneous hemorrhages. The examination of the patient's bone marrow showed diffuse replacement with 64% blasts, with the appearance of plasma blasts. Periodic acid schiff stain was positive in a finely granular pattern. Peroxidase stain was negative. Acid phosphatase and nonspecific esterase stains were brightly positive. Myeloid/erythroid ratio was elevated to 39:1. The bone marrow and peripheral blood findings were diagnostic of acute plasmablastic leukemia. Cytogenetic studies were obtained on this marrow specimen (see below).

The patient began treatment with Alkeran (7.5 mg/day; GlaxoSmith Kline, Middlesex, UK) and prednisolone (50 mg/day) for 7 days. There was clinical improvement, but leukocytosis with circulating blasts, anemia, and thrombocytopenia persisted. Biochemical tests confirmed a gammopathy with total globulins at 45.19 g/L (normal level, 23–35), IgG at 39.33 g/L (normal level, 5–16 g/L), albumin at 21.41 g/L (normal level, 35–50 g/L), and albumin/globulin (A/G) ratio of 0.474 (normal level, 1.43–1.53). Skull roentgenogram showed multiple round radiolucencies in the calvarium, consistent with the patient's plasma cell malignancy. The patient was again treated with a 7-day course of Alkeran — this time at 10 mg/day — and prednisolone (50 mg/day), as well as two weekly doses of vincristine (2 mg intravenously), leading to improvement in symptoms and CBC.

He was re-admitted 3 weeks later with pain, swelling, proptosis, and hyphema of his right eye due to endophthalmitis. CBC revealed a pancytopenia with 0% blasts. For the first time, the liver appeared to be enlarged, extending 4 cm below the right costal margin. Intravenous antibiotics and transfusion of packed red cells resulted in resolution of the endophthalmitis and pancytopenia (WBC 6.3 $\times$ 10^9/L, Hg 8.1 g/L, and platelet count 280 $\times$ 10^9 with 0% blasts). The patient underwent further chemotherapy with weekly vincristine times three and daily dexamethasone, and was discharged home in a markedly improved clinical condition. Unfortunately, the patient's health soon deteriorated, and he died at home 1 month later.

3. Materials, methods, and results

3.1. Cytogenetic and SKY studies

Cytogenetic studies were performed on a bone marrow specimen obtained at diagnosis. Multiple chromosomal abnormalities were identified by G-banding analysis. SKY, performed as described previously [10] on a total of 20 cells from the same specimen, revealed a hypodiploid karyotype with 45 chromosomes and the following clonal rearrangements: der(8;20)t(8;20)(p11.2;p?12), der(11)t(11;14)(q13;q32), and der(14)t(8;14)(p?;p11.2)t(11;14)(q13;q32) (Fig. 1A).

3.2. FISH studies

FISH was performed to confirm the breakpoints involved and to detect possible immunoglobulin and c-*MYC* abnormalities [11]. For the heavy chain immunoglobulin locus (*IgH*), three-color FISH was performed using a fluoroisothyocyanate (FITC)-labeled CH probe (14q32) that detects the strong enhancer elements Eα1 and Eα2 of the constant region, a TRITC-labeled variable region VH probe (14q32) that is located 100 kilobases (kb) from the 14q telomere, and a Cy-5–labeled whole chromosome 14 painting probe (WCP14). For the λ light chain immunoglobulin locus (*Igλ*), three-color FISH was performed using an FITC-labeled Cλ probe (22q11.2) that detects an enhancer element located 3' of the constant region, a TRITC-labeled variable region Vλ probe (22q11.2), and a Cy-5–labeled WCP22 probe. For the *MYC* locus, two-color FISH was performed using a TRITC-labeled c-*MYC* probe (8q24) and a Cy-5–labeled WCP8 probe.

A balanced reciprocal translocation was detected for the *IgH* locus. The normal chromosome 14 had colocalizing CH and VH signals, a derivative 14 had CH and no VH signals, and a derivative 11 had colocalizing CH and VH signals (Fig. 1B). In addition, there were two distinct sets of CH signals on derivative 14, which indicated that a portion of 14q32 was duplicated (Fig. 1D). The *Igλ* locus appeared

Fig. 1. Composite karyotype from SKY and FISH studies: 45,XY,+der(6)del(6)(p?11.2)del(6)(q?15)[1],der(8;20)t(8;20)(p11.2;p?12)[19],der(11)t(11;14)(q13;q32)[18],der(14)t(8;14)(p?;p11.2)t(11;14)(q13;q32)[19],-17[4],-19[4],-20[19],-21[3].ishder(8)t(8;20)(WCP8+,WCP20+,MAFBtel+mafb,MAFBcen+,MYC+),der(11)t(11;14)(WCP11+,WCP14-,CCND1-,CH14+,VH14+),der(14)t(8;14)t(11;14)(WCP11+,WCP14+,CCND1+,CH14+,VH14-)[cp19]. (A) SKY karyotype from a single cell demonstrating 45,XY,der(8)t(8;20)(p11.2;p?12),der(11)del(11)(q13),der(14)t(8;14)(p?;p11.2)t(11;14)(q13;q32),-20. (B) Metaphase chromosomes hybridized with CH (*IgH* constant region; green), VH (*IgH* variable region; red), and WCP14 (purple). There is one normal 14 with colocalizing CH and VH, one copy of der(14) with CH but no VH, and one copy of der(11) with CH and VH. (C) Metaphase chromosomes from Fig. 1B were stripped and rehybridized with c-*MYC* (red), *MAFB* (red), and WCP8 (purple). There is one normal 8 with c-*MYC*, the der(8;20) with c-*MYC* and *MAFB*, and a normal 20 with *MAFB*. The arrow indicates the location of CH seen in Fig. 1B. (D) Metaphase chromosomes hybridized with CH (green). Arrow indicates duplication of the CH signal on der(14). (E) Metaphase chromosomes hybridized with CH (green), *CCND1* (red), and WCP11 (purple). Arrows indicate colocalizing CH and *CCND1* on der(14) on metaphase chromosomes and in an interphase nucleus. (F) Cartoon demonstrating probe locations at their normal chromosome sites and in rearrangements involving chromosomes 8 (orange), 11 (blue), 14 (yellow), and 20 (purple).

to be normal, with two copies of colocalizing Cλ and Vλ signals (data not shown). The c-*MYC* locus did not show any apparent abnormalities: one copy of c-*MYC* was on the normal chromosome 8 and a second copy was on der(8;20)t(8;20)(p11;p?12) (Fig. 1C).

To confirm the target gene for the *IgH* translocation, we hybridized metaphase chromosomes with the FITC-labeled CH probe, the TRITC-labeled cyclin D1 (*CCND1*) probe (11q13), and the Cy-5-labeled WCP11 probe. One copy of *CCND1* was on the normal chromosome 11 and a second copy colocalized with CH on the der(14)t(8;14)(p?;p11.2)t(11;14)(q13;q32) (Fig. 1E). The der(11)t(11;14) lacked the *CCND1* signal but showed a CH signal.

To clarify the breakpoint on der(8;20), we hybridized metaphase chromosomes with the TRITC-labeled *MAFB* probe (20q11), FITC-labeled probes that are centromeric (cen) and telomeric (tel) to *MAFB* (located about 1Mb from *MAFB*), and Cy-5-labeled WCP20. All three *MAFB* probes colocalized, indicating that the breakpoint occurred on 20p: Fig. 1C demonstrates that *MAFB* is in the arm of chromosome 20 that is not involved in the translocation.

The final karyotype, combining the results of SKY and FISH studies, was 45,XY,+der(6)del(6)(p?11.2)del(6)(q?15)[1],der(8)t(8;20)(p11.2;p?12)[19],der(11)t(11;14)(q13;q32)[18],der(14)t(8;14)(p?;p11.2)t(11;14)(q13;q32)[19],-17[4],-19[4],-20[19],-21[3].ishder(8;20)t(8;20)(WCP8+,WCP14-,WCP20+,MAFB tel+,MAFB cen+,MYC+)der(11)t(11;14)(WCP11+,WCP14-,CCHD1-,CH14+,VH14+),der(14)t(8;14)(11;14)(WCP11+,WCP14+,CCND1+,CH14+,VH14-) [cp19]/46,XY,+der(6)del(6)(p?11.2)del(6)(q?15),-15[1] (Fig. 1). The probe locations at their normal chromosome sites and in rearrangements are shown in Fig. 1F. The colored ideograms from this case, along with a clinical summary, are displayed on the National Cancer Institute's and National Center for Biotechnology Information's SKY/M-FISH and comparative genomic hybridization database (http://www.ncbi.nlm.nih.gov/sky/skyweb.cgi).

4. Discussion

Several recent studies have compared the cytogenetic findings in plasma cell leukemia and multiple myeloma [2,3,8,9]. They reported that cytogenetic changes are more frequent in PCL than in MM, and while MM is more likely to show a hyperdiploid karyotype and recurrent trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21 [6,12], PCL is characterized by a hypodiploid pattern, a higher prevalence of complex structural rearrangements, and a higher incidence of deletions of 13q14 (60% or greater) and 17p13.1 (*TP53*). The hypodiploid pattern includes pseudodiploidy, hypodiploidy, and hypotetraploidy; the latter represents 4n duplications of pseudodiploid or hypodiploid karyotypes [13]. In both diseases, deletion of chromosomes 13 and 17 are associated with a poor prognosis, and c-*MYC* involvement is associated with disease progression [5].

Immunoglobulin H (*IgH*) translocations are a hallmark of plasma cell dyscrasias and involve five recurrent chromosomal loci: 11q13 (*CCND1*), 6p21 (*CCND3*), 4p16 (*FGFR3* and *MMSET*), 16q32 (c*MAF*), and 20q11 (*MAFB*) [5]. These translocations are a result of errors in developmentally regulated *IgH* switch recombination, as well as a break in the constant region (CH) with insertion of the 3' α-1 enhancer adjacent to the oncogene, resulting in its overexpression. The CH illegitimate switch recombinations are most likely very early events in tumorigenesis, occurring at the time of physiological *IgH* switch recombination [11]. Gabrea et al. [11] showed a similar cryptic rearrangement in the U266 myeloma cell line in which the CH juxtaposed to *CCND1* on a der(11). In their interphase FISH study of 40 PCL and 247 stage III MM patients, Avet-Loiseau et al. [3] found a higher incidence of *IgH* involvement in PCL than in stage III MM (80 vs. 60%), specifically t(11;14) (33 vs. 16%) and t(14;16) (13 vs. 1%); the incidence of t(4;14) was identical (12%).

The present PCL case, with a survival of 5 months, demonstrated advanced-stage disease with hypodiploidy, two novel chromosomal rearrangements [der(8;20)t(8;20) and der(14)t(8;14)t(11;14)], and monosomy 17 in a minority of cells. There were no trisomies of the odd-numbered chromosomes and no abnormalities of chromosome 13. As a result of translocations into the *IgH* locus, the *CCND1* target gene from 11q13 was juxtaposed to strong enhancer elements at 14q32, resulting in overexpression of the gene. The complex derivative 14 contained a duplication of 14q32 and a translocation of 8p to the 14p arm. We postulate that the formation of the reciprocal t(11;14) was the initial event, and that subsequent remodeling generated the new derivative. A der(14)t(8;14)t(11;14) has not been reported previously [14] and neither has the der(8;20). Aberrations of 20p have been reported in 30 cases of plasma cell neoplams, including 4/40 PCL cases [3], none of which involved translocations with chromosome 8 (as determined by the Cancer Chromosomes database http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=cancerchromosomes). Neither the c-*MYC* nor the *MAFB* oncogenes were involved with an *IgH* translocation in this case (Fig. 1C).

This case demonstrates the usefulness of combining standard cytogenetics with FISH and SKY techniques to fully characterize complex rearrangements and cryptic translocations that can lead to oncogenic amplification and a rapidly progressive clinical course. Such information may be useful in designing future therapies directed at specific molecular targets in this malignancy.

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